



TARAXACUM OFFICINALE AND CIRSIUM ARVENSE METHANOLIC EXTRACTS AMELIORATE OXIDATIVE STRESS AND LIPID PROFILE IN HIGH FAT DIET-INDUCED HYPERCHOLESTEROLEMIC RATS

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Abstract:

The present study was designed to evaluate the effect of *Taraxacum officinale* and *Cirsium arvense* to ameliorate oxidative stress and lipid profile. For this purpose, methanol extract of the *Taraxacum officinale* and *Cirsium arvense* was prepared. Total phenol and total flavonoid content of the extract was performed. In vitro, the antioxidant activity of the plants was evaluated by ABTS, FRAP and DPPH assay. Then, 6 weeks old 40 albino rats were taken for in vivo study. Rats were divided into 5 groups; control group (CON), hypercholesterolemic group and Saline solution (HC+Saline), hypercholesterolemia and Atorvastatin (Hc+As), hypercholesterolemia and *Cirsium arvense* (Hc+Cs) and hypercholesterolemia and *Taraxacum officinale* (HC+To). Chow maintenance diet was served to the control group throughout the experiment. All the groups were fed on high-fat diet and treatments for 42 days. At the end of the experiment, animals were slaughtered to collect blood and serum for analysis of oxidative stress indicators, lipid profile and liver enzyme levels. Results showed the presence of TPC and TFC in both plants *Taraxacum officinale* and *Cirsium arvense*. Methanol extracts also showed antioxidant activity in ABTS, FRAP and DPPH assay. In experimental model, results showed that total antioxidant capacity (TAC), Paraoxonase activity, arylesterase activity, catalase, and SOD were significantly increased in Hc+To and Hc+Cs group as compared to the other groups. TOS and MDA were significantly decreased in treatment Hc+To and Hc+Cs group as compared to the other groups. Total cholesterol, LDL and triglyceride levels was also reduced in Hc+To and Hc+Cs group as compared to the other groups. The extract improved the HDL level in Hc+To and Hc+Cs groups. The study concluded that *Taraxacum officinale* and *Cirsium arvense* has the ability to improve the oxidative stress and lipid profile.

Keywords: *Taraxacum officinale*, *Cirsium arvense*, Hypercholesterolemia, Antioxidant activity, Paraoxonase activity

Introduction:

A prominent known risk factor for cardiovascular disease is hypercholesterolemia,¹ and reducing low-density lipoprotein cholesterol (LDL-C) levels has been demonstrated to significantly decrease

cardiovascular risk in both primary and secondary prevention. There have been reports of varying rates of cardiovascular disease occurrences in patients with equal total cholesterol levels. It is generally known that factors such as food, smoking, physical inactivity, high blood pressure, obesity, diabetes, and thrombogenicity all contribute significantly to the risk of cardiovascular disease.²

Increased cholesterol levels in hypercholesterolemia cause changes in the physical characteristics of cell membranes, which may make it easier for reactive oxygen species (ROS) to escape from the mitochondrial electron transport chain or for NADPH oxidase to get activated.² These reactive free radicals cause the cell membrane's lipids to peroxide, creating lipid peroxide radicals and more free radicals.^{3,4} It is well established that oxidative stress contributes to cardiovascular diseases and that lipid oxidation plays a part in certain of its consequences.⁵ The gold standard of therapy, statins have been shown to reduce LDL-C and lower the risk of CVD.⁶ However, there is a lot of variation in how each person responds to statins⁷ and many people who are at risk for CVD do not meet their LDL-C objectives.^{8,9} Moreover, a number of patients exhibit intolerance to statins, typically as a result of weakness and myalgias.¹⁰ Due to these variables, research is needed to create novel, complementary treatments with beneficial side effects that would increase our capacity to meet LDL-C targets and lower cardiovascular disease risk.¹¹

Taraxacum officinale (Dandelion) is an edible plant belonging to the family Asteraceae.¹² It most likely began in Europe and expanded gradually to North America, Asia, and eventually several South American nations.¹³ *Taraxacum officinale* roots include minerals, sugars (such as glucose, fructose, and sucrose), vitamins, choline, pectin, and mucilage in addition to carbohydrates (such as inulin), carotenoids (such as lutein), fatty acids (such as myristic acid), and other nutrients.¹⁴ Flowers and leaves have higher polyphenol concentrations, mainly hydroxycinnamic acid derivatives and flavonoids than stems.¹⁵⁻¹⁷ They have strong antioxidant and hypocholesterolemic qualities. Although flavonoids decrease the generation of ROS and nitrogen by decreasing NO synthase and COX-2 protein expression,^{18, 19} hydroxycinnamic acid derivatives produce antiradical and protective actions against oxidative processes.¹⁵ The Plant has immunoprotective, antibacterial, antifungal, hepatoprotective, anti-colitis, antiviral, antioxidant antiarthritic, anticancer, antiobesity, and antidiabetic effects. *Cirsium arvense* is a perennial plant that also belongs to Asteraceae family.²⁰ The plant is used as food and flavoring agent. It is a source of flavonoids, tannins, sterols, triterpenes, phenolic acids, and coumarins.^{20, 21} It has medicinal uses for the treatment of tuberculosis, peptic ulcer metrorrhagia, eye infections, skin sores, bleeding piles, syphilis gonorrhoea, epistaxis, and leukaemia.²² The present study was designed to determine the *Taraxacum officinale* and *Cirsium arvense* effect to ameliorate oxidative stress and lipid profile in High fat induced hypercholesterolemic rats.

Material and Methods:

Plant procurement

Taraxacum officinale and *Cirsium arvense* was purchased from a local Lahore, Pakistan, market. Subsequently, knowledgeable botanists from Government College University Faisalabad approved it.

Extract Preparation

The extracts were prepared using the extraction method outlined by Mustafa et al.²³ The plant was rinsed with distilled water, dried in the shade, and ground into a powder. The powder (50 g) was then soaked for 72 hours, occasionally shaking and mixing, in methanol (250 mL). The mixture was filtered using filter paper (Whatman No. 1). A rotary evaporator (SCI100-Pro; SCILOGEX, USA) was used to concentrate the filtrates and transfer them to a Petri plate at a temperature of 40 °C. The petri dish was kept at 40 C in an incubator until it was fully dry. The extract was stored at 4°C until further study.

Qualitative phytochemical study

Phytochemical studies of extracts were analyzed qualitatively by using standard procedures as stated by Singh and Bag²⁴ to identify the major phytochemical components.

Quantitative phytochemical study

Total phenolic content (TPC)

Plant extract (10 μ l, 1 mg/mL) was diluted in 200 μ l of 2.5% Na₂CO₃ and 100 μ l of Folin-Ciocalteu reagent. According to Kainama et al.²⁵ descriptions, TPC in the extracts was calculated using the mean of the gallic acid standard curve. After incubation for 60 minutes at room temperature, absorbance (A) was measured using a Biolab 310 biochemistry analyzer at 760 nm.

Total flavonoids content (TFC)

According to Nisar et al.²⁶ TFC were determined using quercetin (Q) as a benchmark. In a nutshell, 100 μ l of plant extract (1 mg/mL) was combined with 1 mL of distilled water (DW). After incubating at room temperature for 5 minutes, 125 litres of aluminium chloride and 75 litres of 5% sodium nitrite were added, and the mixture was incubated for an additional 6 minutes. Eventually, 125 μ l of 1M sodium hydroxide were combined, and DW was used to create an absolute volume of 2.5 mL. A chemical analyzer was used to quantify the absorbance at 540 nm.

In vitro antioxidant assay

Ferric reducing antioxidant potential (FRAP) assay (μ mole Fe²⁺/g DW)

The technique which Nisar et al.²⁷ described to calculate FRAP was used. The 5 μ l sample was combined with 3.995 mL of working solution (1 volume of 10 mM 2, 4, 6-tripyridyl-s-triazine in 40 mM HCl, 300 mM acetate buffer (10 volumes), and 20 mM ferric chloride (1 volume). At 593 nm, the absorbance was measured.

ABTS assay (Trolox equivalent/g DW)

The ABTS test was conducted as Nisar et al.²⁷ have shown. K₂S₂O₈ solution and a 7-mM ABTS solution in distilled water were combined in a 1:1 ratio to create the ABTS combination (2.5 mM). The resultant liquid was further diluted with methanol to achieve an absorbance of 0.7 at 734 nm. Then, 5 μ l of each plant extract solution and ABTS solution were combined (3.995mL). After 30 minutes of incubation at room temperature, the absorbance at 734 nm was measured.

DPPH scavenging activity

The plant extracts were diluted with dimethyl sulfoxide (DMSO) at a concentration of mg/mL. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was dissolved in methanol (0.025 g/L). 585 μ l of the DPPH working solution and 5 μ l of the sample solution were mixed. After a 20-min incubation time at room temperature, the absorbance was measured at 515 nm with a chemical analyzer (Biolab-310). The DPPH scavenging activity percentage was estimated using the equation $A_0 - A_1 / A_0 \times 100 = \%$. Scavenging action for DPPH A1 is the absorbance of the sample, and A0 is the absorbance of the control (the sample was altered with DW).²⁷

In vivo study

Hypercholesterolemia induction

6 week old 40 albino rats were taken. The animals were already acclimated to the experimental animal station, where the temperature was kept at $26 \pm 2^\circ\text{C}$ and the relative humidity ranged from 40 to 60 percent. Rats were divided into 2 groups; control group of 8 rats and a group of 32 hypercholesterolemic rats. Chow maintenance diet was served to the control group. The hypercholesterolemic group was given a diet that causes hypercholesterolemia.²⁸ The composition of the hypercholesterolemic diet was starch (74%), protein (10%), Oil (10%), vitamin and mineral

mixture (4%), cholic acid (0.5%), and cholesterol (1.5%) as described by Nisar et al.²⁸ This dietary regimen was followed by the second group for 24 days to cause hypercholesterolemia.

Experimental design

Five groups of rats were formed after hypercholesterolemia was induced, one of which was the initial control group (CON), and the hypercholesterolemic rats were split into four groups; hypercholesterolemic group (HC+Saline), hypercholesterolemia and Atorvastatin (Hc+As), hypercholesterolemia and *Cirsium arvense* (Hc+Cs) and hypercholesterolemia and *Taraxacum officinale* (HC+To). The standard chow maintenance diet was served to the CON group at a rate of 48 g/kg body weight. The High fat diet was also administered in the treatment groups at a rate of 48 g/kg body weight. The Hc+As was fed on Atorvastatin (1.5mg/kg b.w), Hc+Cs was fed on *Cirsium arvense officinale* (400 mg/kg body weight), HC+To was fed *Taraxacum officinale* (400 mg/kg body weight) with the High fat diet for 42 days, whereas the HC+Saline continued on the High fat diet and saline solution. Animals were slaughtered at the end of the experiment to collect blood and serum for analysis of oxidative stress indicators, lipid profile and liver enzyme levels.

Oxidative stress indicator

Total antioxidant capacity

The procedure defined by Nisar et al.²⁸ was used to ascertain the serum samples' total antioxidant capacity (TAC). The final concentration, which was expressed as millimoles of vitamin C equivalent per litre, was estimated by calibrating vitamin C standards at concentrations of 0.3, 0.6, 0.9, 1.2, and 1.5 mmol/L. A 660-nm monochromatic wavelength was chosen.

Total oxidative stress

The method defined by Nisar et al.²⁸ was used to ascertain the serum samples' total oxidative stress (TOS). From the standard curve, the TOS of the samples was calculated in equivalent to H₂O₂ standards (6.25, 3.12, 1.56, 0.78, and 0.39 μ mol/L).

Paraoxonase activity

Using the approach established by Anwar et al.²⁹, PON1 activity was estimated. The enzyme's activity was calculated using the reference formula and expressed in Unit/min/l. The paraoxon hydrolysis rate was steady for up to 6 minutes, and the intra assay Variation was less than 10%.

Arylesterase activity

Using a method and formula that have been previously reported, the arylesterase activity per minute was determined.²⁹ This assay's minimal detection limit ranged from 40 to 55 kU per minute per litre.

Catalase activity

By combining 100 ml of serum sample with 1000 ml of substrate (649 mmol/ml H₂O₂ in 59 mmol/l phosphate buffer saline), the catalase activity was determined as described by Mustafa et al.³¹ One millilitre of distilled water was substituted for the substrate in the control test tube and one hundred millilitres of distilled water for the serum in the standard test tube. Ammonium molybdate was used to stop the reaction after it had been incubated for 3 minutes at 37°C (32.4mM). Using Bio-lab 310, the optical density was estimated at 374 nm against a blank.

Malondialdehyde level and superoxide dismutase activity

500 μ l of the serum and 2.5 ml of a 10% thiobarbituric acid solution were combined for MDA, and the tube was then placed in a boiling water bath for 15 minutes. It was then spun at 3000 g for 5 minutes while being cooled in cold water. 2 ml of the supernatant was added to 1 ml of 0.67% TBA solution in a test tube, and the mixture was then heated in a boiling water bath for an additional 15 minutes before being cooled in tap water.³⁰ The Biolab 310 was used to measure the absorbance at

532 nm against blank. Superoxide dismutase were analyzed by using ELISA kits manufactured by Elabscience, USA.

Serum Lipid Profile

The serum levels of total cholesterol, triglycerides, and HDL cholesterol were measured using commercially available colorimetric test kits made by Sigma-Aldrich, Germany. For the assay technique of serum total cholesterol, serum triglycerides, and serum HDL cholesterol level assessment, the instructions supplied in the kits were followed. The Friedrick equation was used to calculate serum LDL-cholesterol levels.

Serum Liver enzyme level

Sigma diagnostics Co. provided a commercially available liquiform technique kit that was used to test the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum.

Statistical analysis

The statistical analysis was performed using SPSS software, version 23. The impact of the *Taraxacum officinale* and *Cirsium arvense* therapy on the examined parameters was established by using one-way analysis of variance. Every dataset was statistically shown as mean + standard error. Differences in group means were analyzed using the Tukey post hoc test. At $p < 0.05$, the level of significance was actively taken into account.

Results:

Qualitative phytochemical study

The presence or absence of phytochemicals, including carbohydrates, reducing sugar, phenols, tannins, alkaloids, flavonoids, steroids, proteins and terpenoids in methanol extract of *Taraxacum officinale* (MthTo) and *Cirsium arvense* (MthCs) is described in Table.1.

Total phenol and flavonoids content

Results indicated that the methanol extracts of *Taraxacum officinale* and *Cirsium arvense* possesses the TPC (29.68 ± 0.43 mgGAE/g, 26.70 ± 0.87 mgGAE/g respectively) and TFC content 22.71 ± 0.03 mgQE/g, 23.37 ± 2.6 mgQE/g respectively) (Table. 1).

In vitro antioxidant assay

Results of the antioxidant assays are expressed in Table.2. The results showed *Taraxacum officinale* and *Cirsium arvense* possess reducing potential of Fe^{3+} into Fe^{2+} (812.74 ± 5.40 $\mu\text{moleFe}^{2+}/\text{g}$, 753.77 ± 9.61 $\mu\text{moleFe}^{2+}/\text{g}$ respectively) and scavenging ABTS radicals (566.78 ± 8.33 $\mu\text{MTE}/\text{g}$, 662.67 ± 5.36 $\mu\text{MTE}/\text{g}$ respectively) assay (Table.2). MthTo and MthCs also showed antioxidant activity against DPPH method with lowest inhibitory concentration ($\text{IC}_{50} = 0.65 \pm 0.009$ mg/mL, 0.62 ± 0.24 mg/mL respectively).

In vivo study

Oxidative stress indicator

Results showed that total antioxidant capacity (TAC) was significantly increased in Hc+To (2.503 ± 0.12 mM/L) and Hc+Cs (2.10 ± 0.09 mM/L) groups as compared to the Hc+Saline group (0.995 ± 0.08 mM/L) and Hc+As group (1.976 ± 0.04 mM/L). TOS was significantly decreased in treatment Hc+To group (18.761 ± 0.98 uM/L) and Hc+Cs (19.78 ± 1.6 uM/L) as compared to the Hc+Saline group (33.217 ± 1.4 uM/L) and Hc+As group (25.32 ± 0.78 uM/L) as shown in Table.3. Paraoxonase and arylestrase activity was also improved in the Hc+To group (29.13 ± 0.81 U/L, 150.45 ± 5.2 KU/L respectively) and Hc+Cs group (26.32 ± 1.2 U/L, 147.91 ± 2.4 KU/L respectively) as compared to the other experimental groups (Table.3). Similar pattern was seen in catalase and SOD activity. Catalase and SOD was significantly increased in the Hc+To group (31.425 ± 2.3 KU/min,

10.12±0.91 ng/ml respectively) and Hc+ Cs group (28.098±0.9 KU/min, 9.025±0.45 ng/ml) as compared to the other groups. MDA was significantly decreased in Hc+To group (0.785±0.09 mMole Vit. C Equiv. /L) and Hc+Cs group (0.886±0.09 mMole Vit. C Equiv. /L) as compared to the other groups as shown in Table.3.

Serum lipid profile

Results showed that total cholesterol, LDL and triglyceride levels was significantly decreased in Hc+To group (204±4.82 mg/dl, 83± 3.09 mg/dl, 168± 2.10 mg/dl respectively) and Hc+Cs group (212±4.01 mg/dl, 86±2.21 mg/dl, 160±3.13 mg/dl) as compared to the Hc+Saline group (310±22.12 mg/dl, 130±12.93 mg/dl, 223± 19.15 mg/dl respectively). HDL level was significantly increased in Hc+To group (124±1.61 mg/dl) and Hc+Cs (122±2.24 mg/dl) as compared to the Hc+Saline group (71±0.15 mg/dl) as shown in Figure.1.

Serum Liver enzymes

Results showed that ALT and AST levels was significantly decreased in Hc+To group (21.22±2.1 U/L, 44.26± 1.3U/L respectively) as compared to the Hc+Saline group (72.22± 3.2 U/L, 68.98± 3.4 U/L respectively) as shown in Figure.2.

Discussion

Increased cholesterol levels in hypercholesterolemia cause changes in the physical characteristics of cell membranes,³¹ which may make it easier for reactive oxygen species (ROS) to escape from the mitochondrial electron transport chain or for NADPH oxidase to get activated.³² These reactive free radicals cause the cell membrane's lipids to peroxide, creating lipid peroxide radicals as well as more free radicals.³³ Hypercholesterolemia and oxidative stress may lead to many diseases including cardiovascular diseases, atherosclerosis, hypertension and diabetes.³⁴⁻³⁸ The present study was designed to evaluate the effect of *Taraxacum officinale* and *Cirsium arvense* to ameliorate lipid profile and oxidative stress. For this purpose, methanolic extract of the plants was prepared. Total flavonoids and phenol contents of the extract were evaluated. The result showed that the methanolic extract of *Taraxacum officinale* and *Cirsium arvense* has the TPC (29.68±0.43 mgGAE/g, 26.70±0.87 mgGAE/g respectively) and TFC content 22.71±0.03 mgQE/g, 23.37±2.6 mgQE/g respectively). Khan et al.³⁹ also described the presence of TPC content in the hydroalcoholic extract of *Taraxacum officinale*. In another study, Chon et al.⁴⁰ showed the presence of TPC and TFC in the methanol extract of different parts of *Taraxacum officinale*. Hossain et al.⁴¹ described that *Cirsium arvense* has high total phenol contents. Shamsutdinova et al.⁴² defined different flavonoids content in the methanolic extract of *Cirsium arvense*. In the present study, FTIR analysis of the *Taraxacum officinale* showed the presence of Hydroxy group, Methylene, Alkenyl, Aromatic ring, Vinyl, Phenol or tertiary alcohol, Primary & secondary alcohol, aliphatic fluoro compound, Peroxides, aliphatic chloro compounds, and aliphatic bromo compounds. FTIR analysis of the *Cirsium arvense* showed the presence of Hydroxy group, Methyl, Alkenyl, Aromatic ring, Carbonate ion, Nitrate ion, Organic phosphates and Silicateion.

In vitro, antioxidant activity results in the present study showed that *Taraxacum officinale* and *Cirsium arvense* has antioxidant capacity. *Taraxacum officinale* and *Cirsium arvense* showed antioxidant activity in ABTS (566.78±8.33 µMTE/g, 662.67±5.36 µMTE/g respectively), FRAP (812.74±5.40 µmoleFe²⁺/g, 753.77±9.61 µmoleFe²⁺/g respectively), and DPPH IC₅₀ = 0.65±0.009 mg/mL, 0.62±0.24 mg/mL respectively).assays. Ivanov,⁴³ revealed that the ethanol extract of the plant leaves has antioxidant activity against DPPH (136.3 mM TE/ g DW) and FRAP (131.5 mM TE / g DW) assay. Bioactive components i.e hydroxycinnamic acids and sesquiterpene lactones of the plant also showed antioxidant activity in the DPPH assay.⁴⁴ In the present study, in vivo study was designed for the further confirmation of the antioxidant activity of the *Taraxacum officinale*.

6 week old 40 albino rats were taken. Rats were divided into 2 groups; control group of 8 rats and a group of 32 hypercholesterolemic rats. Chow maintenance diet was served to the control group. The hypercholesterolemic group was given a high fat diet that causes hypercholesterolemia. Hypercholesterolemic rats were split into four treatment groups; HC+Saline, Hc+As, Hc+To and HC+Cs. Antioxidants parameter results showed that total antioxidant capacity (TAC), Paraoxonase activity, arylesterase activity, catalase, and SOD were significantly increased in Hc+To and Hc+Cs group as compared to other groups. TOS and MDA were significantly decreased in treatment Hc+To and Hc+Cs group as compared to the Hc+Saline group. In the previous study, Sumanth and Rana⁴⁵ described that the alcoholic extract of *Taraxacum officinale* improved the SOD, catalase, glutathione, and peroxidase levels significantly and reduced lipid peroxidation in CCl₄-induced toxic rats. The result of the previous study supports our present study results. However, in vivo antioxidant activity of *Cirsium arvense* was not described in previous studies.

The present study results also revealed that the plant extracts also improved the lipid profile in the high fat induced hypercholesterolemic rats. Total cholesterol, LDL and triglyceride levels were significantly decreased in Hc+To (204±4.82 mg/dl, 83± 3.09 mg/dl, 168± 2.10 mg/dl respectively) and Hc+Cs (212±4.01 mg/dl, 86±2.21 mg/dl, 160±3.13 mg/dl) as compared to the other experimental groups. HDL level was significantly increased in Hc+To group (124±1.61 mg/dl) and Hc+Cs (122±2.24 mg/dl) as compared to the other experimental groups. Choi et al.⁴⁶ conducted a study on cholesterol-fed rabbits. The results of the study exposed that *Taraxacum officinale* improved the lipid profile in cholesterol-fed rabbits. In the present study, both plants also improved the AST and ALT levels in Hc+To and Hc+Cs group. You et al.⁴⁷ revealed that the *Taraxacum officinale* reduced the ALT and AST levels in alcohol induced oxidative stress model. In another study, the *Taraxacum officinale* also showed hepatoprotective activity against CCl₄ induced toxic model.⁴⁸

Conclusion

The methanol extract of the *Taraxacum officinale* and *Cirsium arvense* has total phenol content and total flavonoid content. The in vitro and in vivo antioxidant analysis showed that both plant extracts have the strongest ability to reduce oxidative stress. In terms of improving lipid profile, hypercholesterolemic activity was also shown by the methanol extract of the plants. So, it is concluded that *Taraxacum officinale* and *Cirsium arvense* tend to improve lipid profile and oxidative stress.

Conflict of Interest

No conflict of interest

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Table. 1 Qualitative Phytochemical study of *Taraxacum officinale* and *Cirsium arvense*

Compounds	Test	MthTo	MthCs
Carbohydrate	Benedict's test	-	++
Reducing sugar	Fehling's test	-	-
Flavonoids	Alkaline reagent test	+++	+++
Alkaloids	Hager's test	+++	+++
Tannins	Lead acetate test	+	-
Phenols	Lead acetate test	+++	+++
Protein	Xanthopectic test	-	-
Terpenoids	Salkowski's test	+	++
Steroids	Salkowski's test	++	+

Highly positive; +++, moderate positive; ++, weak positive +, nodetection;-

Table.2 Total phenol content, total flavonoid content and in vitro antioxidant activity of methanol extract of *Taraxacum officinale* and *Cirsium arvense*

Parameters	<i>Taraxacum officinale</i> (MthTo)	<i>Cirsium arvense</i> (MthCs)
TPC	29.68±0.43 mgGAE/g	26.70±0.87 mgGAE/g
TFC	22.71±0.03 mgQE/g	23.37±2.61 mgQE/g
FRAP Assay	812.74±5.40 µmoleFe ²⁺ /g	753.77±9.61 µmoleFe ²⁺ /g
ABTS Assay	566.78±8.33 µMTE/g	662.67±5.36 µMTE/g
DPPH scavenging activity	IC ₅₀ = 0.65±0.009 mg/mL	IC ₅₀ = 0.62±0.24 mg/mL

TPC, Total phenol content; **TFC**, Total flavonoid content; **FRAP**, Ferric reducing antioxidant potential; **ABTS** Assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **DPPH** scavenging activity, 2, 2-diphenyl-1-picrylhydrazyl scavenging activity

Table 3. Oxidative stress indicators in different experimental groups

	TAC (mM/L)	TOS (μM/L)	PON (U/L)	ARY (KU/L)	SOD (ng/ml)	MDA(pM/dl)	Catalase (KU/min)
CON	1.546±0.16	22.67±2.2	20.69±1.2	139.724±4.7	9.4738±0.78	1.199±0.2	19.0512±1.9
Hc+Saline	0.995±0.08	33.217±1.4	16.785±1.9	120.1238±4.9	6.0349±0.24	1.9701±0.3	12.1109±0.76
Hc+As	1.976±0.04	25.32±0.78	18.23±0.78	127.12±3.7	9.984±0.17	0.954±0.1	25.031±0.89
Hc+Cs	2.10±0.09	19.78±1.6	26.32±1.2	147.91±2.4	9.025±0.45	0.886±0.09	28.098±0.9
Hc+To	2.5038±0.12	18.761±0.98	29.13±0.81	150.4501±5.2	10.1187±0.91	0.7858±0.09	31.4259±2.3

TAC, Total antioxidant capacity; **TOS**, Total oxidative stress; **PON**, Paraoxonase; **ARY**, aryletrase; **SOD**, Superoxide dismutase; **MDA**, Malanodialdehyde.

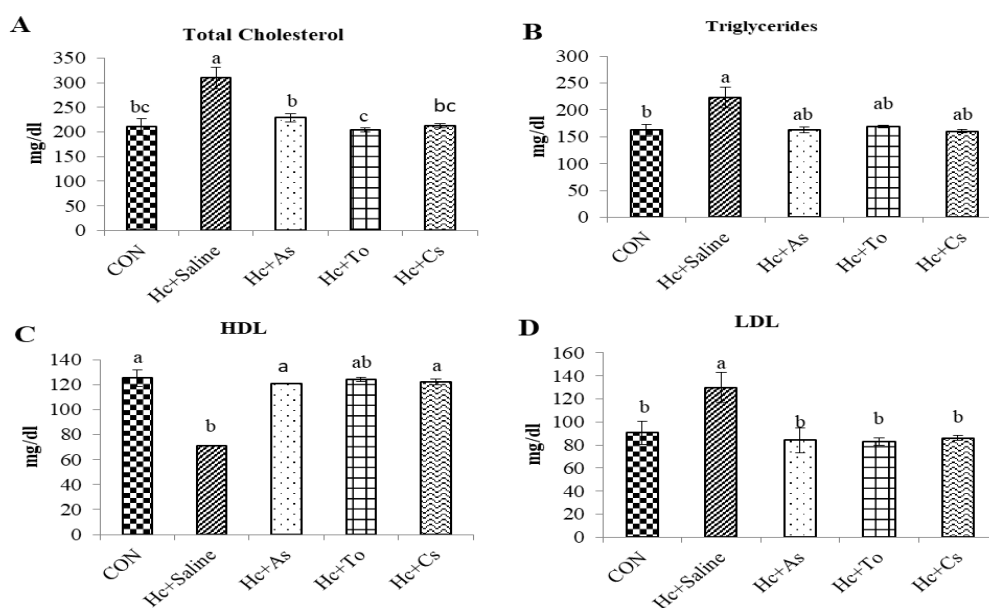


Figure 1. Serum lipid profile in different experimental groups

HDL, High density lipoprotein; **LDL**, Low density lipoprotein

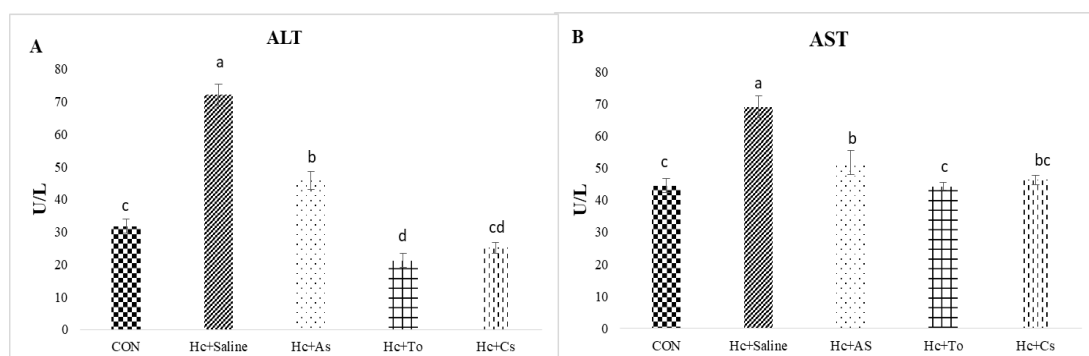


Figure 2. Serum Liver enzyme levels in different experimental groups

AST, Aspartate aminotransferase; **ALT**, Alanine aminotransferase